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EXPRESS MAIL LABEL NO. EL628755847US

534 Rec'd PCT/PTO 12 JUL 2000

Optical Arrangement in the Illumination Beam Path of a Microscope

This invention relates to an optical arrangement in the illumination beam path of a microscope, in particular of a confocal laser microscope.

BACKGROUND OF THE INVENTION

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In confocal laser microscopy, it has for some time been part of the existing art to expand the laser beam (which of itself is Gaussian) in the illumination beam path, by way of a suitable optical system, in such a way that the entry pupil of the respective objective or objectives usable there is fundamentally overilluminated. The degree of overillumination is an important design parameter. Overillumination of the entry pupil on the one hand provides homogeneous illumination thereof, the purpose being to guarantee the theoretical resolution, in particular in the case of objectives having different apertures. On the other hand, especially in the case of objectives with a small entry pupil, overillumination of the entry pupil results in considerable losses of excitation light. Such losses of excitation light are, however, not acceptable in applications where performance reserves in the excitation light are low.

The Leica TCS laser scanning microscope, for example, in which a fixed expansion optical system is provided, has become known from practical use. The diameter of the laser beam expanded therein is approximately 25 mm ($1/e^2$ value) at the microscope objective.

The divergence of the laser light and thus the illumination of the aperture that is effective for the excitation light can be controlled by modifying the size of the excitation pinhole. Reference is made in this context, solely by way of example, to G.J. Brakenhoff et al. in Confocal Microscopy Handbook, J. Pawley, ed., 1994, pp. 87-91.

A PL APO 40X/1.25 objective, for example, has an entry pupil approximately 12 mm in diameter. A PL APO 100X/1.4 objective, on the other hand, has an entry pupil only 5 mm in diameter. As a result, in the latter the excitation light is lost by a factor of $(12/5)^2 = 5.76$ due to unnecessary overillumination.

Even if the beam path before the excitation pinhole is otherwise unchanged, the pinhole transmission in proportion to the area of the pinhole is characterized by corresponding light losses at small diameters. This, too, is unacceptable for practical use.

SUMMARY OF THE INVENTION

It is thus the object of the present invention to describe an optical arrangement in the illumination beam path of a microscope in which optimum illumination is guaranteed while reducing losses of excitation light.

The optical arrangement of the generic type according to the present invention achieves the aforementioned object by way of ~~the features of Claim 1. According to this,~~ an optical arrangement ~~of this kind is~~ characterized by an illumination optical system, arranged in the illumination beam path, to modify the illumination diameter.

According to the present invention, it has been recognized that the illumination diameter of the illumination beam should be more or less exactly adapted to the entry pupil of the objective in question in order to avoid light losses. Achieving this requires an illumination optical system, arranged in the illumination beam path, with which the illumination diameter can be modified or adapted. In this manner, light losses such as those of the existing art mentioned above can be at least largely avoided.

Concretely, the illumination optical system provided according to the present invention could be embodied as an arrangement of replaceable fixed optics. When an

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objective is replaced, the fixed optics in the illumination beam path would correspondingly need to be replaced, so that the illumination diameter is matched to the entry pupil of the respective objective.

In very particularly preferred fashion, the illumination optical system comprises a variable optical system, preferably operating steplessly, so that it is unnecessary to replace fixed optics in the illumination beam path. The variable optical system can be a preferably motorized zoom optical system, which in turn can be embodied as an ordinary zoom optical system such as is used, for example, in commercially available video cameras.

For simple and optimum adaptation of the illumination diameter to the entry pupils of multiple objectives, an automatic adjustment system could be provided. Concretely, the modification in the illumination diameter could be matched to the entry pupils of predefined objectives, preferably arranged in a revolving nosepiece, the modification or adaptation being accomplished automatically depending on the particular objective being used (corresponding to the position in the nosepiece).

In terms of concrete potential applications of the optical arrangement according to the present invention, it is conceivable for the illumination optical system to be arranged downstream from a point light source or an optical fiber. The illumination optical system could be embodied as a parallelizing optical system with ~~a fixed focal intercept but variable focal length,~~ the beam diameter being adaptable to the entry pupil of the objective.

It is also conceivable for the illumination optical system to be embodied as an expanding optical system for a preferably directly coupled-in laser beam. ~~In this context the beam could be variably expandable in accordance with the ratio f_1/f_2 of the focal lengths.~~

It has already been explained above by way of example that overillumination has been accepted in the existing art, especially when objectives have small entry pupils. The edge illumination in such cases, however, was certainly good. In order to promote edge illumination when an arrangement according to the present invention is used, it is advantageous, in particular with large entry pupils, if the illumination optical system comprises a further optical component that influences or favors edge illumination, the overillumination known from the existing art being avoided in any case. An optical component of this kind could be embodied as an additional lens, as an annular stop, or as a holographically generated optical element, the principal result thereof being that the ordinarily Gaussian laser beam is expanded in the edge regions. For example it would be possible thereby, especially in the case of confocal laser scanning microscopy, to achieve a constant intensity distribution over the entire entry pupil without causing substantial overillumination of the entry pupil of the objective. An intensity profile deviating therefrom may also be advantageous for a specific application.

It is furthermore conceivable to provide, in the case of the arrangement according to the present invention, an additional input for feeding in a further light source, this preferably involving the coupling-in of a laser light beam. With no modification of the actual illumination beam path, this laser light beam could be adaptable to the entry pupil of the objective, thus also making possible in this context an optimization of the laser light beam with no adaptation of the actual illumination beam path.

Lastly, an arrangement of the aforesaid kind could advantageously be used in multiphoton laser scanning microscopy or for multiphoton excitation.

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There are various ways of advantageously embodying and developing the teaching of the present invention. Reference is made, for that purpose, on the one hand to the claims ~~which follow Claim 1~~, and on the other hand to the explanation below of three

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exemplary embodiments of the invention with reference to the drawings. In conjunction with the explanation of the preferred exemplary embodiments of the invention, a general explanation is also given of preferred embodiments and developments of the teaching. ~~The drawings show~~

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Fig 1 shows, in a schematic depiction, a first exemplary embodiment of an optical arrangement according to the present invention in the beam path of a confocal scanning microscope, a point light source being provided as the light source and the scanning microscope being depicted, for the sake of simplicity, merely schematically by way of its components;

Fig. 2 shows, in a schematic depiction, a second exemplary embodiment of an optical arrangement according to the present invention in the beam path of a confocal scanning microscope, an optical fiber being provided as the light source and the scanning microscope being depicted, for the sake of simplicity, merely schematically by way of its components; and

Fig. 3 shows, in a schematic depiction, a third exemplary embodiment of an optical arrangement according to the present invention in the beam path of a confocal scanning microscope, a laser light source or laser beam being provided as the light source and the scanning microscope being depicted, for the sake of simplicity, merely schematically by way of its components.

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DETAILED DESCRIPTION OF THE INVENTION

FIGS. 1 through 3 each show an optical arrangement in the illumination beam path 1 of a confocal scanning microscope, the scanning microscope as a whole being depicted merely schematically for the sake of simplicity.

While in FIG. 1 a point light source 2 is depicted (symbolically) as the light source, in FIG. 2 the light is coupled in via an optical fiber 3. In the exemplary embodiment in

FIG. 3, a laser beam 4 or a parallel light beam of an alternative/conventional light source is coupled via a lens 5 directly into illumination beam path 1.

According to the present invention, in all three exemplary embodiments (FIGS. 1, 2, and 3) an illumination optical system 6 is arranged in illumination beam path 1. This illumination optical system 6 serves to modify illumination diameter 7, thus making it possible for illumination diameter 7 to be adapted to the (symbolically depicted) entry pupil 8 of objective 9.

For better comprehension, the Figures show not only illumination beam path 1 as far as object 10, but also a scanner 12 and a beam combiner 11 arranged in illumination beam path 1.

A pinhole optical system 14 and a detection pinhole 15 (depicted schematically) are arranged in detection beam path 13.

In the exemplary embodiments depicted in FIGS. 1 and 2, illumination optical system 6 is embodied as a steplessly operating variable optical system. More precisely, in this case it is a motorized zoom optical system that, however, is shown merely symbolically by way of a shiftable lens 16. Concretely, what is being discussed here is an ordinary zoom optical system such as is known from video cameras.

In the embodiment depicted in FIG. 3, illumination optical system 6 is preceded by a lens 5 into which laser beam 4 is directly coupled.

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027 To avoid repetition, reference is made to the general portion of the Specification regarding further features not evident from the Figures.

Parts List

1	Illumination beam path
2	Point light source
3	Optical fiber
4	Laser beam
5	Lens (downstream from the laser beam)
6	Illumination optical system
7	Illumination diameter
8	Entry pupil of the objective
9	Objective
10	Object
11	Beam combiner
12	Scanner
13	Detection beam path
14	Pinhole optical system
15	Detection pinhole
16	Lens (of the illumination optical system)